

CLAIMS

1. A compound having bone stimulatory activity in mammals, the compound being derived from a polypeptide having the charge pattern of the amino acid side chain charges provided by the amino acid sequence corresponding to SEQ ID NO:9 wherein the amino acid sequence can have up to 35 consecutive amino acids selected from the sequence corresponding to SEQ ID NO:1.
2. The compound of claim 1 wherein the compound includes a backbone which is substantially isosteric with that provided by the peptide backbone of the amino acid sequence corresponding to SEQ ID NO:9, wherein the amino acid sequence can have up to 30, 25, 20, 15, or 10 consecutive amino acids selected from the sequence corresponding to SEQ ID NO:1, and wherein the compound can be a polypeptide.
3. The compound of claim 1 wherein the charge pattern of the compound consists essentially of that provided by the amino acid sequence corresponding to SEQ ID NO:9.
4. The compound of claim 3 having the charge pattern and spacing of that provided by the amino acid sequence corresponding to SEQ ID NO:9.
5. The compound of claim 1, including substitutions of the sequence corresponding to SEQ ID NO:9 which retain bone stimulatory activity in mammals.
6. The compound of claim 5, wherein the compound is a polypeptide and a said sequence from which the polypeptide is derived consists of up to 25 consecutive amino acids selected from the sequence corresponding to SEQ ID NO:1 and conjugates of one or more selected sequences; and wherein a said sequence from which the polypeptide is derived can consist of up to 20 consecutive amino acids selected from the sequence corresponding to SEQ ID NO:1 and conjugates of one or more selected sequences; and wherein a said sequence from which the polypeptide is derived can consist of up to 15 consecutive amino acids selected from the sequence corresponding to SEQ ID NO:1 and conjugates of one or more selected sequences; and wherein a said sequence from which the polypeptide is derived can consist of up to 10 consecutive amino acids selected from the sequence corresponding to SEQ ID NO:1 and conjugates of one or more selected sequences.
7. The compound of claim 3, wherein the compound is a polypeptide having an amino acid sequence selected from that corresponding to SEQ ID NO:9; SEQ ID NO:25; SEQ ID NO:26; SEQ ID NO:27; SEQ ID NO:39; SEQ ID NO:40; SEQ ID NO:41; SEQ ID NO:42; SEQ ID NO:43; SEQ ID NO:44; SEQ ID NO:45 and SEQ ID NO:46, including substitutions which retain bone stimulatory activity in mammals.
8. A compound having bone stimulatory activity in mammals, the compound having the charge distribution of the side chain charges provided by the amino acid sequence corresponding to SEQ ID NO:9 and having up to about 83% sequence homology with SEQ ID NO:1.
9. The compound of claim 8, wherein the compound is a polypeptide up to 30 amino acids in length, and conjugates thereof, wherein the compound can have up to about 69% sequence homology with SEQ ID NO:1; or wherein the compound is a polypeptide up to 25 amino acids in

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length, and conjugates thereof, wherein the compound can have up to about 55% sequence homology with SEQ ID NO:1; or wherein the compound is a polypeptide up to about 20 amino acids in length, and conjugates thereof, and wherein the compound can have up to about 42% sequence homology with SEQ ID NO:1; or wherein the compound is a polypeptide up to about 15 amino acids in length, and conjugates thereof, and wherein the compound can have up to about 28% sequence homology with SEQ ID NO:1; or wherein the compound is a polypeptide up to about 10 amino acids in length, and conjugates thereof.

10. A polypeptide having bone stimulatory activity in mammals, the polypeptide having the charge distribution of the side chain charges provided by the amino acid sequence identified as SEQ ID NO:9 and including at least one non-conservative substitution at position number 2, 3, 6 or 9; wherein the polypeptide can have an amino acid sequence derived from SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:42, or SEQ ID NO:43, and substitutions thereof which retain bone stimulatory activity in mammals.

11. A polypeptide comprising an amino acid sequence corresponding to SEQ ID NO:1 or SEQ ID NO:3 with (a) from one to about four 4 amino acids deleted from the N-terminus of SEQ ID NO:1 or SEQ ID NO:3, (b) one to about 22 amino acids deleted from the C-terminus of SEQ ID NO:1 or SEQ ID NO:3, or both (a) and (b); or a functionally equivalent homologue which promotes bone growth in mammals wherein the polypeptide can comprise an amino acid sequence corresponding to SEQ ID NO:4 with (a) up to about four 4 amino acids deleted from the N-terminus of SEQ ID NO:4, (b) up to about 16 amino acids deleted from the C-terminus of SEQ ID NO:4, or both (a) and (b); or a functionally equivalent homologue which promotes bone growth in mammals; and wherein the polypeptide can comprise an amino acid sequence corresponding to SEQ ID NO:5 with (a) up to about four 4 amino acids deleted from the N-terminus of SEQ ID NO:5, (b) up to about 11 amino acids deleted from the C-terminus of SEQ ID NO:5, or both (a) and (b); or a functionally equivalent homologue which promotes bone growth in mammals; and wherein the polypeptide can comprise an amino acid sequence corresponding to SEQ ID NO:6 with (a) up to about four 4 amino acids deleted from the N-terminus of SEQ ID NO:6, (b) up to about 5 amino acids deleted from the C-terminus of SEQ ID NO:6, or both (a) and (b); or a functionally equivalent homologue which promotes bone growth in mammals; and wherein the polypeptide can comprise an amino acid sequence corresponding to SEQ ID NO:7 with (a) up to about four 4 amino acids deleted from the N-terminus of SEQ ID NO:7, (b) up to about 1 amino acids deleted from the C-terminus of SEQ ID NO:4, or both (a) and (b); or a functionally equivalent homologue which promotes bone growth in mammals; and wherein the polypeptide can comprise an amino acid sequence corresponding to SEQ ID NO:8 with up to about four 4 amino acids deleted from the N-terminus or a functionally equivalent homologue which promotes bone growth in mammals

12. A polypeptide up to about 20 amino acids in length comprising an amino acid sequence corresponding to SEQ ID NO:9, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53,

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or SEQ ID NO:54, or a functionally equivalent homologue thereof which promotes bone growth in mammals.

13. A polypeptide of claim 12, having a charge pattern of the amino acid side chain charges provided by the amino acid sequence corresponding to SEQ ID NO:9.

14. A polypeptide of claim 13 comprising an amino acid sequence corresponding to SEQ ID NO:29 or SEQ ID NO:54.

15. A polypeptide of claim 14 consisting essentially of an amino acid sequence corresponding to SEQ ID NO:29 or SEQ ID NO:54, including protected derivatives thereof.

16. A polypeptide of claim 12, wherein the polypeptide is synthetic and the amino acid sequence has a molecular weight limited to the range of from about 1000 to 4000, or of from about 1000 to 3000, or from about 1000 to 2000, or from about 1000 to about 1500.

17. A first polypeptide having a sequence up to about 30 amino acids in length which polypeptide is sufficiently duplicative of a second polypeptide comprising an amino acid sequence corresponding to SEQ ID NO:1 or SEQ ID NO:3 with (a) from one to about four 4 amino acids deleted from the N-terminus of SEQ ID NO:1 or SEQ ID NO:3, (b) one to about 22 amino acids deleted from the C-terminus of SEQ ID NO:1 or SEQ ID NO:3, or both (a) and (b), or a functionally equivalent homologue thereof, such that the first polypeptide is encoded by a DNA that hybridizes under stringent conditions with DNA encoding the second polypeptide.

18. A polypeptide which is a conservatively substituted variant of a polypeptide of claim 10.

19. A synthetic polypeptide having in vivo bone stimulatory activity in mammals and which increases mineral content in bones of mammals, having an amino acid sequence which is at least about 19% conserved in relation to the amino acid sequence identified as SEQ ID NO:1 and having at least one amino acid deleted therefrom, or a functionally equivalent homologue; or a said synthetic polypeptide having an amino acid sequence which is at least about 22% conserved in relation to the amino acid sequence identified as SEQ ID NO:1 and having at least one amino acid deleted therefrom; or a said synthetic polypeptide which is at least about 25% conserved in relation to the amino acid sequence identified as SEQ ID NO:1 and having at least one amino acid deleted therefrom; or a said synthetic polypeptide having an amino acid sequence which is at least about 28% conserved in relation to the amino acid sequence identified as SEQ ID NO:1 and having at least one amino acid deleted therefrom.

20. A polypeptide of claim 19 having at least six amino acids deleted from said sequence, or having at least sixteen amino acids deleted from said sequence, or having at least twenty-one amino acids deleted from said sequence, or having at least twenty-six amino acids deleted from said sequence.

21. A polypeptide of claim 20 wherein the polypeptide has a molecular weight in the range of from about 1000 to 4000, or from about 1000 to 3000, or from about 1000 to 2000, or from about 1000 to 1500.

22. A first polypeptide comprising a sequence of amino acids sufficiently duplicative of a second polypeptide comprising an amino acid sequence of claim 19 such that the first polypeptide is

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encoded by a DNA that hybridizes under stringent conditions with DNA encoding the second polypeptide.

23. A chimeric bone stimulating factor comprising a polypeptide of claim 6.

24. A method of increasing bone growth in a mammal by administering a therapeutically effective amount of a polypeptide of claim 6.

25. A method of increasing bone growth in a mammal by administering a therapeutically effective amount of a compound of claim 1.

26. The use of a polypeptide of claim 6, for the treatment of osteoporosis.

27. The use of a compound of claim 1, for the treatment of osteoporosis.

28. The use of a polypeptide of claim 6, to promote bone growth in a mammal.

29. The use of a compound of claim 1, to promote bone growth in a mammal.

30. A diagnostic kit for determining the presence of a polypeptide of claim 6, comprising an antibody to a said polypeptide linked to a reporter system wherein the reporter system produces a detectable response when a predetermined amount of the polypeptide and the antibody are bound together.

31. A diagnostic kit for determining the presence of a compound of claim 1, comprising an antibody to a said compound linked to a reporter system wherein the reporter system produces a detectable response when a predetermined amount of the compound and the antibody are bound together.

32. An isolated DNA fragment which encodes the expression of any of the polypeptides of claim 6, and DNA which differs from the fragment due to the degeneracy of the genetic code.

33. A vector comprising a DNA sequence which encodes the expression of any of the polypeptides of claim 6.

34. A process for producing a polypeptide of claim 6, which comprises:

a) preparing a DNA fragment containing a nucleotide sequence which encodes said polypeptide;

b) incorporating said DNA fragment into an expression vector to obtain a recombinant DNA fragment which contains said DNA fragment and is capable of undergoing replication;

c) transforming a host cell with said recombinant DNA fragment to isolate a transformant which can express said polypeptide; and

d) culturing said transformant to allow the transformant to produce said polypeptide and recovering said polypeptide from resulting cultured mixture.

35. A polypeptide having bone stimulatory activity, the polypeptide comprising an amino acid sequence that has $10 + q$ amino acids, wherein, under physiological conditions, residues numbered n , $n + 4$, $n + 9$ are positively charged amino acids, residues numbered $n + 3$, $n + 7$ are negatively charged amino acids, wherein the remaining amino acids are nonpolar amino acids or uncharged polar amino acids, wherein n is an integer from 1 to $1 + q$ and q is an integer from 0 to 15; and, wherein the polypeptide includes no more than 25 consecutive amino acids corresponding to the amino acid sequence identified as SEQ ID NO:1.

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36. The polypeptide of claim 35, wherein $q = 10, 5$ or 0 .
37. The polypeptide of claim 35 wherein each of the remaining amino acids is selected from the group consisting of glycine, alanine, valine, isoleucine, serine, threonine, methionine, asparagine and glutamine.
38. The polypeptide of claim 35, wherein the residue numbered $n + 8$ is cysteine, serine, tyrosine or alanine.
39. The polypeptide of claim 35, wherein the residue numbered n is arginine.
40. The polypeptide of claim 35, wherein the residue numbered $n + 1$ is alanine or threonine.
41. The polypeptide of claim 35; wherein the residue numbered $n + 2$ is alanine, asparagine, or glutamine.
42. The polypeptide of claim 35, wherein the residue numbered $n + 3$ is glutamic acid.
- ⁴³ 43. The polypeptide of claim 35, wherein the residue numbered $n + 4$ is histidine.
- ⁴⁴ 44. The polypeptide of claim 35, wherein the residue numbered $n + 5$ is threonine or alanine.
- ⁴⁵ 45. The polypeptide of claim 35, wherein the residue numbered $n + 6$ is glycine or alanine.
- ⁴⁶ 46. The polypeptide of claim 35, wherein the residue numbered $n + 7$ is glutamic acid or aspartic acid.
- ⁴⁷ 47. The polypeptide of claim 35, wherein the residue numbered $n + 9$ is lysine.
- ⁴⁸ 48. The polypeptide of claim 38, wherein the residue numbered $n + 8$ is serine.

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